An Investigator-initiated Study to Assess the Safety and Efficacy of Ingenol Mebutate 0.05% Gel When Used After Cryosurgery in the Treatment of Hypertrophic Actinic Keratosis on Dorsal Hands

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ABSTRACT

Objectives: To evaluate the safety and efficacy of ingenol mebutate 0.05% gel after cryosurgery versus cryosurgery alone for the treatment of hypertrophic and nonhypertrophic actinic keratosis on the dorsal hands. **Design:** Investigator-blinded split arm study. **Setting:** Academic institution. **Participants:** Sixteen subjects with actinic keratoses on dorsal hands. **Results:** There was a mean reduction in the number of hypertrophic actinic keratosis lesions adjusted for baseline in ingenol mebutate-treated versus control group of -4.3 versus -2.8, respectively. There was a mean reduction in the number of non-hypertrophic actinic keratosis lesions in the ingenol mebutate-treated versus control group of -3.8 versus -0.3. **Conclusion:** A statistically significant and clinically meaningful difference in response was demonstrated in favor of ingenol mebutate-treated hands versus controls. No significant increase in local skin responses was noted when applying ingenol mebutate 0.05% gel on the same day as cryosurgery. Trial registry: ClinicalTrials.gov, NCT02251652.
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Actinic keratosis (AK) occurs on chronically sunlight-exposed skin and represents one of the most common diseases treated by dermatologist.[1] It is believed that the dysplasia arises from ultraviolet radiation causing deoxyribonucleic acid pyrimidine covalent dimers, mutations in the p53 tumor suppressor gene, and local and systemic immunosuppresion.[2,3] AKs may be considered as pre-malignant lesions or incipient squamous cell carcinoma (SCC).[4] Although the risk for progression from an individual AK to SCC is low, the risk over a lifetime can become substantial as the number of AKs increases, thus affecting treatment considerations.[5] Importantly, the area surrounding AKs has endured the same ultraviolet radiation exposure, resulting in "field cancerization" and the risk of subclinical AKs transforming into clinical AKs or possibly SCCs.

Current treatment modalities for AKs include light therapy, topical therapy, cryosurgery, and excisional surgery. The efficacy of cryosurgery is directly related to the freeze time; however, a longer duration of ice-crystal formation may result in greater discomfort and localized skin reactions, such as hypopigmentation. Furthermore, subclinical lesions in the surrounding area are not addressed by targeted cryosurgery. Treated lesion clearance rates at three months post-treatment after double-freeze thaw cryosurgery has been reported to be roughly 76 to 88 percent, although new lesions in the treatment field were not included.[6,7] There is limited information on long-term AK clearance rates after cryosurgery, with reports differing in their methods of calculation. In one investigation comparing cryosurgery, imiquimod 5% cream, and 5-fluorouracil 5% cream, at 12 months after cryosurgery, 28 percent of patients showed complete clearance of baseline lesions that had undergone cryosurgery, but only four percent showed complete clearance of the treatment field.[8] Thus, new

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TABLE 1. Study inclusion and exclusion criteria	
INCLUSION	
1	Subject at least 18 years of age
2	Subjects must be in good general health as confirmed by the medical history
3	Subjects must be able to read, sign, and understand the informed consent
4	Prior to cryosurgery, subjects have at least 3 hypertrophic actinic keratoses on each dorsal hand
5	Subject must be willing to forego any other treatments on the dorsum of the hands, including tanning bed use and excessive sun exposure while in the study
6	Subject is willing and able to participate in the study as an outpatient, making frequent visits to the study center during the treatment and follow-up periods and to comply with all study requirements including concomitant medication and other treatment restrictions
7	If subject is a female of childbearing potential she must have a negative urine pregnancy test result prior to study treatment initiation and must agree to use an approved method of birth control while enrolled in the study
EXCLUSION	
1	Subjects with a history of melanoma anywhere on the body
2	Subjects with an unstable medical condition as deemed by the clinical investigator
3	Subjects with nonmelanoma skin cancer on the dorsum of the hands
4	Subjects with any dermatologic disease in the treatment area that may be exacerbated by the treatment proposed or that might impair the evaluation of actinic keratoses
5	Subjects who have previously been treated with ingenol mebutate: On the dorsum of the hands in the past 6 months or outside of the study area within the past 30 days
6	Women who are pregnant, lactating, or planning to become pregnant during the study period
7	Subjects who have experienced a clinically important medical event within 90 days of the visit (e.g., stroke, myocardial infarction, etc.)
8	Subjects who have active chemical dependency or alcoholism as assessed by the investigator
9	Subjects who have known allergies to any excipient in the study gel
10	Subjects who are currently participating in another clinical study or have completed another clinical study with an investigational drug or device on the study area within 30 days prior to study treatment initiation
11	Subjects who have received any of the following within 90 days prior to study treatment initiation: • interferon or interferon inducers • cytotoxic drugs • immunomodulators or immunosuppressive therapies (inhaled/intranasal steroids are permitted) • oral or parenteral corticosteroids • topical corticosteroids if greater than 2g/day • any dermatologic procedures or surgeries on the study area (including any actinic keratosis treatments)
12	Subjects who have used any topical prescription medications on the study area within 30 days prior to study treatment initiation

TABLE 2. Study flowchart

ASSESSMENT/PROCEDURE	DAY 1	DAY 4	DAY 8	DAY 15	DAY 29	DAY 57
Informed consent	√					
Inclusion/exclusion criteria	√					
Medical history	√					
Demographic information	√					
Urine pregnancy test (if applicable)	√					
Photography	√	√	√	√	√	√
Lesion count	√	√	√	√	√	√
Local skin reaction assessment	√	√	√	√	√	√
Cryosurgery	√					
Dispense medication	√					
Collect medication		√				
Concomitant medications	√	√	√	√	√	√
Adverse events	√	√	√	√	√	√

treatment methods or combination treatment regimens are needed for improved lesional and field clearance of AKs.

Ingenol mebutate is currently United States Food and Drug Administration-approved for the treatment of non-hypertrophic (non-HT) AKs on the face/scalp (0.015% gel) for three consecutive days, and the chest/extremities (0.05% gel) for two consecutive days.[9] A total of four Phase 3 studies have evaluated the efficacy of ingenol mebutate versus placebo. Two studies (n=547) compared ingenol mebutate 0.015% gel versus vehicle gel applied daily for three consecutive days to a 25cm^2 area of the face and scalp. Relative to vehicle gel, ingenol mebutate 0.015% gel demonstrated higher complete clearance rates (42.4% vs. 3.7%, p<0.001) as well as partial clearance rates (63.9% vs. 7.4%, p<0.001). Two studies (n=458) comparing ingenol mebutate 0.05% gel to vehicle gel applied for two consecutive days to a 25cm^2 area of the trunk and extremities showed higher complete clearance rates (34.1% vs. 4.7%, p<0.001) and partial clearance rates (49.1% vs. 6.9%, p<0.001).

The authors evaluated the efficacy of ingenol mebutate 0.05% gel for the treatment of hypertrophic (HT) AKs on the dorsal surface of hands in a sequential manner with cryosurgery, as no such data exists at this time. We hypothesized that the sequential treatment of AKs with cryosurgery followed by ingenol mebutate would lead to higher clearance rates than cryosurgery alone. Cryosurgery provides short-term efficacy on treated AKs, with ingenol mebutate providing the treatment benefit of an entire field, including subclinical lesions.

Dorsal hands are a common location for AKs, which are oftentimes HT lesions. While it is known that an average patient with AKs has 7.7 lesions, the average number of AKs on dorsal hands has not been studied.[10] Since AKs on dorsal hands are oftentimes HT lesions, their response to topical therapy is less optimal. Sequential therapy with cryosurgery and ingenol mebutate may optimize the treatment of HT and non-HT AKs in this anatomic location.

STUDY OBJECTIVES

The primary objective was to evaluate the safety of cryosurgery in combination with ingenol mebutate on the dorsal surface of hands and compare it to the safety of

cryosurgery alone. The secondary objectives were to evaluate and compare the mean reduction in the number of all AKs (HT and non-HT) on the dorsal hands with the combination of cryosurgery-ingenol mebutate versus cryosurgery alone. The study also aimed to evaluate the number of AKs (both HT and non-HT) before therapy on the dorsal hands.

METHODS

Study approval was obtained from the Institutional Review Board at The Icahn School of Medicine at Mount Sinai, New York, New York. Subjects were recruited from the Faculty Practice and resident clinics at Mount Sinai. The participants were selected according to the inclusion and exclusion criteria of the study protocol (Table 1).

A signed and dated informed consent form, photographic consent, and authorization to use and disclose medical information was obtained prior to performing any study-specific procedures. Seventeen subjects were enrolled in the study; one subject withdrew consent prior to treatment. A total of 16 subjects completed all study procedures.

All qualified and enrolled subjects received cryosurgery (liquid nitrogen) to all HT AKs on both dorsal hands at their baseline visit. The cryosurgery was standardized for each lesion in all treated subjects: Two continuous sprays for five seconds each with a five-second interval between sprays.

Following cryosurgery, subjects were randomized by unblinded site personnel to treat either the right or left dorsal hand with ingenol mebutate 0.05% gel. The study physician was blinded to the treatment arm. Under the supervision of the unblinded site personnel, subjects applied the first dose (a single-unit dose tube of ingenol mebutate 0.05% gel) to the entire randomized dorsal hand within a few minutes after receiving the cryosurgery. Subjects utilized once-daily dosing for two consecutive days; therefore, the second single-unit dose tube was dispensed to the subject to be applied at home the following day. Subjects were verbally instructed and provided with written directions regarding the proper technique and regimen for study gel application as well as possible local skin reactions.

Subjects were subsequently followed after their initial visit on Day 4, Day 8, Day 15, Day 29, and Day 57 (study visits had a two-day window period) (Table 2). At each study visit, the investigator assessed the treatment and immediate surrounding areas to grade the local skin reaction using a standardized scale. At follow-up visits, all AK lesions on both dorsal hands were counted and recorded, and the treatment areas were photographed. Concomitant medications and adverse events were also recorded. Whenever possible, the same qualified blinded evaluator assessed the same subject throughout the study.

TABLE 3. Dorsal hand size for each subject

SUBJECT	SA RIGHT HAND (SQUARE INCHES)	SA LEFT HAND (SQUARE INCHES)
1	17.5	17.5
2	17.5	17.5
3	17	18
4	22.3	21
5	15.8	16
6	18.75	18.75
7	17	18
8	19.5	21.1
9	18.6	18.6
10	25	20.25
11	19.5	21.2
12	18.6	18.6
13	22.3	21
14	20.5	21.3
15	18.75	23.25
16	17.5	17

TABLE 4. Actinic keratosis lesions for study groups at baseline and Day 57

HYPERTROPHIC	INGENOL MEBUTATE		INGENOL MEBUTATE + CRYOSURGERY	
SUBJECT #	BASELINE	DAY 57	BASELINE	DAY 57
1	3	2	4	0
2	7	2	7	3
3	1	1	2	0
4	4	2	3	1
5	5	3	13	4
6	3	2	3	1
7	3	1	4	0
8	3	1	7	0
9	10	5	8	2
10	5	2	8	0
11	5	2	2	0
12	7	3	10	0
13	3	1	5	0
14	5	3	3	1
15	4	1	3	0
16	5	2	5	1

TABLE 5. Comparisons of proportion of responders between treated and control hands

TYPE	OUTCOME	INGENOL MEBUTATE 0.05% GEL	CONTROL
Hypertrophic	Baseline # AKs n Mean Median Min, Max	 16 5.4 (3.14) 4.5 2, 13	 16 4.6 (2.13) 4.5 1, 10
	Change from baseline to Day 57 in # AKs n Mean (SD) LS mean (SE) Median Min, Max p-value vs. control	 16 -4.6 (2.66) -4.3 (0.22) -4.0 -10, -2 <0.0001	 16 -2.5 (1.37) -2.8 (0.22) -2.0 -5, 0
	Percent change from baseline to Day 57 n Mean (SD) LS mean (SE) Median Min, Max p-value vs. control	 16 -86.34 (16.642) -86.1 (4.58) -100.0, -57.1 <0.0001	 16 -51.89 (19.091) -52.1 (4.58) -58.57 -75.0, 0.0
	Proportion of subjects CLEAR on Day 57 **N (%)**	 **9 (56.3%)**	 **0 (0.0%)**
	Proportion of subjects with ≥75% reduction from baseline to Day 57 **N (%)** ***p*-value**	 **11 (68.8%)** **0.0016**	 **1 (6.3%)**
Non-hypertrophic	Baseline n Mean (SD) Median Min, Max	 16 4.4 (3.24) 4.0 1, 13	 16 3.7 (2.27) 3.5 1, 9
	Change from baseline to Day 57 n Mean (SD) LS mean (SE) Median Min, Max *p*-value vs. control	 16 -4.1 (3.38) -3.8 (0.37) -3.0 -13, -1 <0.0001	 16 0.0 (0.00) -0.3 (0.37) 0.0 0.0

RESULTS

There were no serious adverse events reported in this trial. For each subject, the size of dorsal hands was measured (Table 3). The difference from baseline to Day 57 in the number of AK lesions was calculated (Table 4). Mean changes in the number of lesions were compared between treated and control hands across all patients using a mixed effects model adjusted for the baseline number of lesions (Table 5); this model also took the within-patient correlation into account. The analyses for HT and non-HT lesions were performed separately.

The mean number of lesions at baseline was slightly higher in the ingenol mebutate-treated hands versus control (HT: 5.4 vs. 4.6 lesions; non-HT: 4.4 vs. 3.7, respectively). Mean reductions in the number of AK lesions from baseline to Day 57 were calculated based on both simple means and LS-means (LS means were adjusted for number of lesions at baseline). The LS means are preferable as there are slight

TABLE 6. Localized skin reactions for treatment groups						
CRYOSURGERY + INGENOL MEBUTATE 0.05%						
	BASELINE	**DAY 3**	**DAY 7**	**DAY 14**	**DAY 28**	**DAY 56**
Erythema	1.375	2.313	1.733	1.438	0.938	0.375
Flaking	0.313	1.375	1.4	1.25	0.625	0.438
Crusting	0.063	1	1.4	1	0.5	0.125
Swelling	0.563	1.813	0.4	0.125	0.125	0
Vesiculation/ papulation	0.063	1.063	0.333	0.125	0	0
Erosion/ulceration	0.062	0.75	1.133	0.688	0.125	0
CRYOSURGERY ONLY						
	BASELINE	**DAY 3**	**DAY 7**	**DAY 14**	**DAY 28**	**DAY 56**
Erythema	1.375	1.5	1.276	1	0.938	0.25
Flaking	0.313	0.813	1.133	1	0.75	0.375
Crusting	0.063	1	1	0.938	0.5	0.063
Swelling	0.563	1.063	0.333	0.063	0	0
Vesiculation/ papulation	0.063	0.75	0.2	0.063	0	0
Erosion/ulceration	0.062	0.563	0.733	0.625	0.125	0

baseline imbalances that may impact treatment outcomes. The mean reductions in number of HT AK lesions adjusted for baseline in the ingenol mebutate-treated hands versus control were -4.3 versus -2.8, respectively ($p<0.0001$). The mean reductions in number of non-HT AK lesions adjusted for baseline in the ingenol mebutate-treated hands versus control were -3.8 versus -0.3, respectively ($p<0.0001$).

The comparisons of proportion of responders between treated and control hands were performed using a Generalized Estimated Equations (GEE) model taking into account possible within-patient correlation.

The proportion of hands clear of HT AKs (defined as 0 lesions at Day 57) was calculated for ingenol mebutate-treated (56.3%) versus control (0%) hands; a p-value based on the GEE model could not be calculated with 0 responders in the control group.

The proportion of hands clear of non-HT AK (defined as 0 lesions at Day 57) was calculated for ingenol mebutate-treated (75%) versus control (0%) hands; a p-value based on the GEE model could not be calculated with 0 responders in the control group.

Both treatment methods of cryosurgery with or without ingenol mebutate have satisfactory safety profiles. Localized skin reactions were evaluated (Table 6, Figures 1 and 2).

Overall, results showed a highly statistically significant and clinically meaningful difference in response in favor of ingenol mebutate-treated versus control hands. The outcomes were consistently significant and in favor of ingenol mebutate for all definitions of response: mean changes from baseline as well as the proportion of responders defined as clear of AK lesions.

The analyses also demonstrated that reduction in absolute number of lesions from baseline to Day 57 strongly depends on the number of lesions at baseline: The more lesions at baseline the greater reduction at Day 57.

DISCUSSION

Previous studies have examined sequential therapies and their ability to optimize clearance outcomes. The authors' results demonstrate the strong utility of combination therapy with ingenol mebutate and cryosurgery in the treatment of AKs of the dorsal hand. The addition of ingenol mebutate represents an attractive progression given its efficacy and high level of adherence.[11] In the cohort of 16 patients with HT and non-HT AKs, combination therapy with ingenol mebutate and cryosurgery resulted in statistically significant improvement. The study data suggest that ingenol mebutate treatment is successful in AK treatment response, particularly in patients

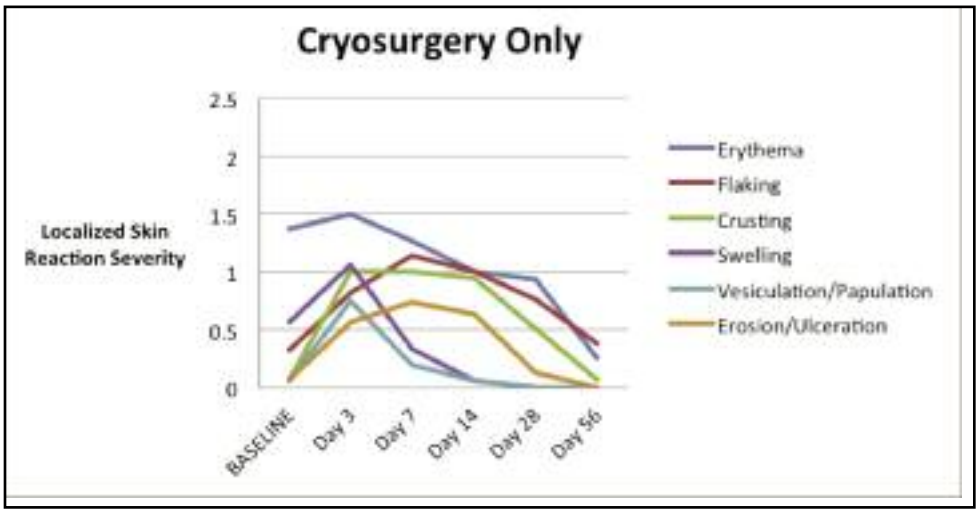

Figure 1. Localized skin reactions for participants receiving cryosurgery alone

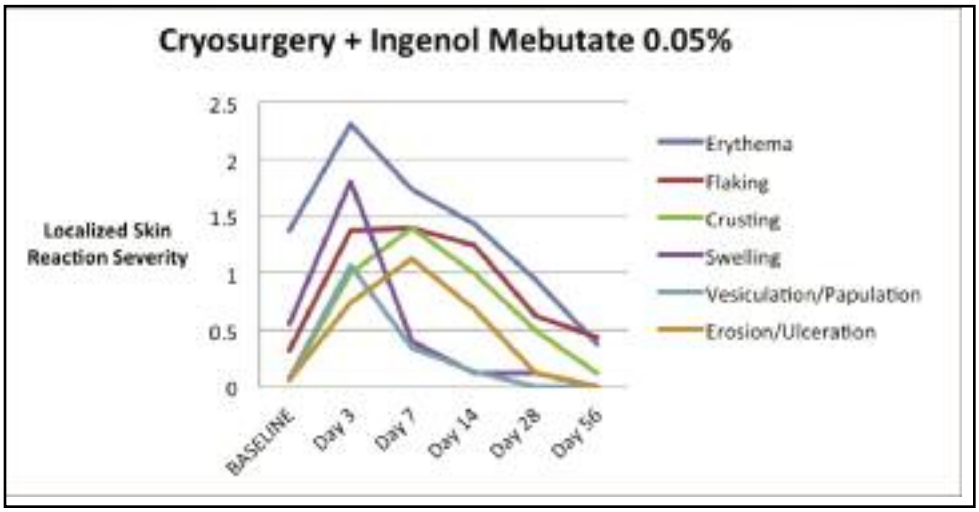

Figure 2. Localized skin reactions for participants receiving cryosurgery with ingenol mebutate 0.05%

with greater numbers of lesions. Moreover, this is the first study to show safety of using ingenol mebutate immediately after cryosurgery. In fact, the authors believe that using ingenol mebutate immediately or soon after cryosurgery may increase the efficacy of the combination treatment.

Study limitations. Results of this study show promise in guiding future AK treatment. Study limitations include small sample size and the fact that LSRs may unblind the investigator. Future improvements in study design may include a longer duration of data collection in order to document the long-term effects of combination therapy and future AK and SCC progression.

Potential further studies to evaluate the efficacy of cryosurgery and ingenol mebutate combination therapies should include risk stratification for patients with and without HT AKs or those with increased numbers of baseline AKs.

REFERENCES

1. Goldberg L and Mamelak AJ. Review of actinic keratosis. Part 1: etiology, epidemiology and clinical presentation. *J Drugs Dermatol.* 2010;9:1125–1132.
2. Stockfleth E, Ferrandiz C, Grob JJ, et al. Development of a treatment algorithm for actinic keratoses: a European consensus. *Eur J Dermatol.* 2008;18(6):651–659.
3. Padilla RS, Sebastian S, Jiang Z, et al. Gene expression patterns of normal human skin, actinic keratosis, and squamous cell carcinoma: a spectrum of disease progression. *Arch Dermatol.* 2010;146(3):288–293.
4. Criscione V, Weinstock M, Naylor M, et al. Actinic keratoses: natural history and risk of malignant transformation in the Veterans Affairs Topical Tretinoin Chemo-prevention Trial. *Cancer.* 2009;115(11):2523–2530.
5. Neidecker MV, Davis-Ajami ML, Balkrishnan R, et al. Pharmacoeconomic considerations in treating actinic keratosis. *Pharmacoeconomics.* 2009;27(6): 451–164.
6. Kaufmann R, Spelman L, Weightman W, et al. Multicentre intraindividual randomized trial of topical methyl aminolaevulinate-photodynamic therapy vs. cryosurgery for multiple actinic keratoses on the extremities. *Br J Dermatol.* 2008;158(5):944–949.
7. Morton C, Cambell S, Gupta G, et al. Intraindividual, right left comparison of topical methyl aminolaevulinate-photodynamic therapy and cryosurgery in subjects with actinic keratoses: a multicentre, randomized controlled study. *Br J Dermatol.* 2006;155(5): 1029–1036.
8. Krawtchenko N, Roewert-Huber J, Ulrich M, et al. A randomised study of topical 5% imiquimod vs. topical 5-fluorouracil vs. cryosurgery in immunocompetent patients with actinic keratoses: a comparison of clinical and histological outcomes including 1-year follow-up. *Br J Dermatol.* 2007;157(Suppl 2):34–40.
9. Lebwohl M, Swanson N, Anderson LL, et al. Ingenol mebutate gel for actinic keratosis. *N Engl J Med.* 2012;366(11):1010–1019.
10. Marks R, Foley P, Goodman G, et al. Spontaneous remission of solar keratosis: the case for conservative management. *Br J Dermatol.* 1986;115(6):649–655.
11. Batalla A, Florez A, Feal C, et al. Clinical response to ingenol mebutate in patients with actinic keratoses. *Actas Dermosifiliogr.* 2015;106(10):e55–e61.